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Highly Variable Pharmacokinetics of Once-Daily Intravenous Busulfan When Combined with Fludarabine in Pediatric Patients: Phase I Clinical Study for Determination of Optimal Once-Daily Busulfan Dose Using Pharmacokinetic Modeling

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Busulfan has a narrow therapeutic range, and in children, pharmacokinetic variability has been found to be high even after the use of intravenous (i.v.) busulfan. Recently, a reduced toxicity myeloablative regimen showed promising results, but the data of busulfan pharmacokinetics in hematopoietic stem cell transplantation (HSCT) using a targeted busulfan/fludarabine regimen in children has not yet been reported. We performed therapeutic drug monitoring (TDM) after once-daily i.v. busulfan combined with fludarabine and analyzed the outcomes. Busulfan (i.v.) was administered once daily for 4 consecutive days. The daily target area under the curve (AUC) was 18,125-20,000 μg*h/L/day (4415-4872 μmol*min/L/day), which was reduced to 18,000-19,000 μg*h/L/day (4384-4628 μmol*min/L/day) after a high incidence of toxicity was observed. A total of 24 patients were enrolled. After infusion of busulfan on the first day, patients showed AUC that ranged from 12,079 to 31,660 μg*h/L (2942 to 7712 μmol*min/L) (median 16,824 μg*h/L, percent coefficient of variation (%CV) = 26.5%), with clearance of 1.74-6.94 mL/min/kg (median 4.03 mL/min/kg). We performed daily TDM in 20 patients, and during the daily TDM, the actual AUC ranged from 73% to 146% of the target AUC, showing high intraindividual variability. The %CV of busulfan clearance of each individual ranged from 7.7% to 38.7%. The total dose of busulfan administered for 4 days ranged from 287.3 mg/m^2 to 689.3 mg/m^2. Graft failure occurred in 3 patients with total AUC less than 74,000 μg*h/L (18,026 μmol*min/L), and 2 patients with relatively high total AUC experienced veno-occlusive disease. Busulfan pharmacokinetics showed high inter- and intraindividual variability in HSCT using a targeted busulfan/fludarabine regimen, which indicates the need for intensive monitoring and dose adjustment to improve the outcome of HSCT. Currently, we are performing a newly designed phase II study to decrease regimen-related toxicities and reduce graft failure by setting an optimal target AUC based on this study.

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KEY WORDS: Busulfan, Fludarabine, Pharmacokinetics, Stem cell transplantation

INTRODUCTION

Busulfan has a narrow therapeutic range. High exposure is associated with systemic toxicity such as veno-occlusive disease (VOD) [1-5], and underexposure is associated with graft failure or relapse [5,6]. After the intravenous (i.v.) formulation was introduced, busulfan pharmacokinetics appeared to be more predictable compared with the previous oral busulfan, especially in adults [7,8]. However, there is still a significant variation of busulfan exposure with the same i.v. dose, and a small proportion of patients will experience toxic exposure [9-12]. Because of this pharmacokinetic variability, therapeutic drug monitoring (TDM) of busulfan and dose adjustment have been recommended to improve

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the clinical outcome of hematopoietic stem cell transplantation (HSCT) [5,12-15].

Many recent reports have shown that once-daily i.v. busulfan could be well tolerated as a conditioning regimen without increasing toxicity [7,8,16,17]. One randomized study demonstrated that the pharmacokinetic profiles and posttransplantation complications are similar between once-daily i.v. busulfan and traditional 4-times-daily i.v. busulfan [18]. In 1 study, once-daily i.v. busulfan was also tolerable in children with limited toxicity, but the graft failure rate was relatively high; that indicated the need for optimization of the busulfan dose using TDM [19].

Recently, a reduced-toxicity myeloablative regimen using busulfan and fludarabine showed promising results [20-23], but the data of busulfan pharmacokinetics when combined with fludarabine in children has not yet been reported. In this study, we performed TDM after once-daily i.v. busulfan combined with fludarabine. We analyzed the pharmacokinetics of busulfan and also evaluated the clinical outcome of HSCT using a targeted busulfan/fludarabine regimen. We also analyzed the effect of a glutathione S-transferase (GST) polymorphism on the interindividual variability of busulfan pharmacokinetics.

MATERIALS AND METHODS

Study Population

Patients undergoing allogeneic HSCT using a busulfan-based conditioning regimen at Seoul National University Children's Hospital were prospectively included in this study from January 2009 to December 2009. This study was approved by the institutional review board of Seoul National University Hospital (H-0809-025-256) and registered at www.clinicaltrials.gov (NCT01018446). Written informed consents were obtained for all patients. During the study period, we decreased target area under the curve (AUC) after interim analysis of 13 patients, and we grouped the patients into group 1 (target AUC 18,125-20,000 μg*h/L/day) and group 2 (target AUC 18,000-19,000 μg*h/L/day).

Transplantation Protocol

The conditioning regimen was composed of busulfan and fludarabine (40 mg/m^2 once-daily i.v. on days −8∼−3). For patients with acute lymphoblastic leukemia, etoposide (20 mg/kg once-daily i.v. on days −4∼−2) was added. Busulfan (i.v.) was administered over 3 hours once daily for 4 consecutive days on days −6∼−3 for a busulfan/fludarabine regimen and on days −8∼−5 for a busulfan/fludarabine/etoposide regimen.

Busulfan was reported to have an age independent correlation between body surface area (BSA) and clearance in previous reports with children [19,24], so busulfan dosing based on the BSA was used in this study. Patients older than 1 year received 120 mg/m^2 as the first dose, and patients younger than 1 year received 80 mg/m^2. From the second day, we used a targeted dose of busulfan according to the TDM results. Bone marrow, mobilized peripheral blood, or cord blood was infused on day 0 of the conditioning regimen.

Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine plus prednisolone or mycophenolate, or tacrolimus plus methotrexate. Patients received low molecular weight heparin with lipo-PGE1 for prophylaxis of VOD. Other supportive care was according to the guidelines for stem cell transplantation in our center [25]. Regimen-related toxicity until 42 days after transplantation was graded according to the NCI Common Toxicity Criteria (NCI-CTC v4.0).

TDM and Dose Adjustment

A specific, accurate, and rapid assay based on high-performance liquid chromatography (Symbiosis Pharma, Spark Holland, the Netherlands) with tandem mass spectrometry was developed and validated for the quantification of busulfan in human plasma using glipizide as the internal standard. Human plasma samples were deproteinated using acetonitrile. Chromatographic separation for busulfan was performed on a Luna C18 column (5 μm, 100 Å, 50 mm × 2 mm; Phenomenex, Torrance, CA) with distilled water containing 0.1% formic acid-acetonitrile as the mobile phase by gradient elution. The flow rate was 0.3 mL/min, and the run time was 4.0 min. Busulfan and glipizide were detected using multiple reaction monitoring in the positive mode, with transitions of *m/z* 264.2 to 150.8 and *m/z* 446.3 to 321.1, respectively. Linear calibration curves were established in the range of 25-5000 ng/mL for busulfan, and the regression correlation coefficients (r) were over 0.9998. The intra- and interbatch accuracy values of quality control samples ranged from 96.5% to 100.4% and 98.5% to 99.9%, and precision variations of quality control samples were <4.3% and 4.7%, respectively.

Blood samplings were taken through the Hickman catheter line, which was not used for busulfan infusion before administration, and at 0, 1, 2, and 4 hours after the end of infusion. AUC and clearance was calculated by 2 compartmental methods using WinNonlin® 5.2.1 (Pharsight, Mountain View, CA). Target AUC was 18,125-20,000 μg*h/L/day (4415-4872 μmol*min/L/day), and dose adjustment was done when AUC was beyond the range. We initially planned to perform TDM on the first and fourth days, as well as the day when a dose adjustment of >25% was needed according to the results of previous study [13]. We changed the design to perform TDM daily, because the actual AUC at the fourth day was higher than the expected

Table 1. Characteristics of Patients

Characteristics	Total (N = 24) No. (%)	Group 1 (N = 13) No. (%)	Group 2 (N = 11) No. (%)
Gender			
Male	11 (45.8)	6 (46.2)	5 (45.4)
Female	13 (54.2)	7 (53.8)	6 (54.5)
Diagnosis			
AML	13 (54.2)	7 (53.8)	6 (54.5)
ALL	7 (29.2)	4 (30.1)	3 (23.1)
MDS	1 (4.2)	1 (7.7)	0 (0.0)
Others*	3 (12.6)	1 (7.7)	2 (18.2)
Transplant type			
Related BMT	1 (4.2)	1 (7.7)	0 (0.0)
Related PBSCT	3 (12.5)	3 (23.1)	0 (0.0)
Unrelated BMT	3 (12.5)	2 (15.4)	1 (9.1)
Unrelated PBSCT	10 (41.7)	5 (38.4)	5 (45.4)
Haploidentical PBSCT	2 (8.3)	1 (7.7)	1 (9.1)
CBT	5 (20.8)	1 (7.7)	4 (30.8)

ALL indicates acute lymphoblastic leukemia; AML, acute myeloid leukemia; BMT, bone marrow transplantation; CBT, cord blood transplantation; MDS, myelodysplastic syndrome; PBSCT, peripheral blood stem cell transplantation.
*Other disease: 1 adrenoleukodystrophy, 1 Wiskott-Aldrich syndrome, 1 Krabbe disease.

AUC with the adjusted dose in 2 patients. The target AUC was reduced to 18,000-19,000 μg*h/L/day (4384-4628 μmol*min/L/day) after we observed a high incidence of toxicity in the interim analysis of 13 patients. From that time, the target AUC at the fourth day was decided as (74,000- cumulative AUC during 3 days) μg*h/L/day.

Genotyping of GST Polymorphism

Seven single nucleotide polymorphisms (GSTA1 promoter −52G>A, −69C>T, −567T>G, −631T>G, GSTP1 313A>G, GSTM1 deletion, and GSTT1 deletion) that have been implicated in the metabolism of busulfan were analyzed by multiplex polymerase chain reaction and single nucleotide polymorphisms genotyping as previously described [26].

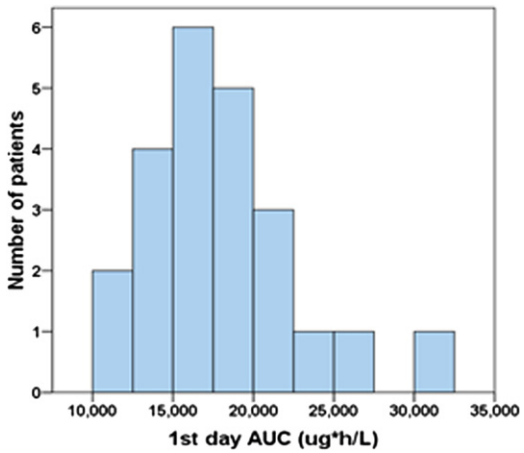

Figure 1. Variability of the first day AUC. Patients showed AUC that ranged from 12,079 to 31,660 μg*h/L (median 16,824 μg*h/L) after infusion of 120 mg/m^2 busulfan on the first day.

Statistics

Differences between means in continuous variables were calculated with the Student t test. The paired t test was used to compare the clearance of the first day and the last day. Inter- and intraindividual variability of busulfan pharmacokinetics was assessed by calculating the percent coefficient of variation (%CV), given as the standard deviation divided by the mean, multiplied by 100. The Kaplan-Meier method and log-rank univariate comparisons were used to estimate the cumulative incidence of toxicities. SPSS version 17.0 (SPSS, Inc., Chicago, IL) was used for all statistical analyses, and statistical significance was accepted when $P < .05$.

RESULTS

Characteristics of Patients

A total of 24 patients were enrolled for this study. The clinical characteristics of the patients are summarized in Table 1. The median age was 9.3 years (range: 0.9-18.1 years), and median BSA was 1.07 m^2 (0.48-1.89 m^2).

Interindividual Variability

In 23 patients, except 1 patient under 1 year who received 80 mg/m^2 of busulfan as the first dose, the AUC ranged from 12,079 to 31,660 μg*h/L (2942 to 7712 μmol*min/L) (median 16,824 μg*h/L, %CV = 26.5%), with clearance of 1.74-6.94 mL/min/kg (median = 4.03 mL/min/kg) after infusion of 120 mg/m^2 busulfan on the first day (Figure 1). In 12 patients, the busulfan dose was increased 1.07-1.53 times compared with the first dose on the second day, and a dose reduction of 0.58-0.95 times compared with the first dose was made in 8 patients. The total dose of busulfan ranged from 287.3-689.3 mg/m^2 (Table 2).

Intraindividual Variability

The third and the fourth patients received the same dose of busulfan after the target AUC was achieved, but they unexpectedly showed an increased AUC (27,753 and 26,060 μg*h/L/day) on the fourth day. After that, daily TDM was performed in 20 patients. Even after the daily dose adjustment, AUC was highly variable among the days (Figure 2). The %CV of busulfan clearance of each individual ranged from 7.7%-38.7% (median = 14.3%).

During the daily TDM and dose adjustment, the actual AUC ranged from 73%-146% of the target AUC. Over 10% of the differences in the actual AUC were observed 39 times (66.1%). Higher than expected AUC (111.4%-145.7%) was observed 23 times (39.0%) with decrease of clearance (68.6%-90.0% compared with the prior day), and lower AUC (73.4%-89.9%) with increased clearance

Table 2. Interindividual Variability of Busulfan Pharmacokinetics

	Total (N = 24)	Group 1 (N = 13)	Group 2 (N = 11)
Dose modification at the second day			
Decreased, n (%)	8 (33.3)	4 (30.8)	4 (36.4)
Increased, n (%)	12 (50.0)	6 (46.2)	6 (54.5)
Total dose of busulfan			
Median, mg/m^2	467.0	470.8	451.1
Range, mg/m^2	287.3-689.3	287.3-569.4	339.3-689.3

(111.1%-136.1% compared with the prior day) was seen 16 times (27.1%). Clearances on the last day were significantly different from those of the first day (P = .001) (Figure 3).

Effect of GST Polymorphism on Busulfan Pharmacokinetics

Although there was no statistically significant difference, the first day AUC had a tendency to be high in patients with the GSTA1 *A/*B genotype or the GSTT1 null genotype (Figure 4). The first day AUC was 16,035 ± 4789 μg*h/L in patients having both the GSTA1 *A/*A and GSTT1 present genotype and 19,146 ± 4474 μg*h/L in the other patients (P = .128).

Effect of Total AUC on Clinical Outcome

Graft failure occurred in 3 patients (1 cord blood transplantation [CBT] and 2 T cell–depleted haploidentical transplantations), with total AUC of 72,300, 72,752, and 73,822 μg*h/L (17,612, 17,722, and 17,983 μmol*min/L). Treatment-related mortality occurred in 4 patients. Causes of treatment-related mortality were adenoviral pneumonia in 1 patient, acute GVHD (aGVHD) with infection in 1 patient, and chronic GVHD (cGVHD) with infection in 2 patients. VOD developed in 2 patients, with total AUC of 79,469 and 81,367 μg*h/L (19,358 and 19,821 μmol*min/L).

Grade III/IV hepatic toxicities were more common in patients whose total AUC was over 77,000 μg*h/L (18,757 μmol*min/L) (P = .006) and in patients of group 1 (P = .007) (Figure 5). The incidence of aGVHD or cGVHD was not different according to the total AUC group.

DISCUSSION

Inter- and intraindividual variability of busulfan pharmacokinetics after oral administration is well known, and it is explained by the difference of absorption. After development of i.v. busulfan, the inter- and intraindividual variability was decreased less than that of the oral drug [7,8,27-29]. However, there was still significant pharmacokinetic variability in many studies, indicating the need for TDM and dose adjustment, even after using i.v. busulfan [12]. Nath et al. showed that there was considerable inter- and intraindividual variability when using i.v. busulfan as a single daily dose. In that study, the %CV of busulfan clearance (l hour^{-1} kg^{-1}) in 40 children was 35%, and they observed and predicted that AUC values deviated from each other by 20%-44% in a subset of patients [10].

Our data also showed high interindividual variability with the 26.5% of %CV of the first day AUC. We analyzed several factors, including age, sex, BSA, body weight, and diagnosis as influencing factors, but they did not have any influence on the interindividual variability. Busulfan is metabolized primarily through the liver by conjugation to reduced glutathione, which is

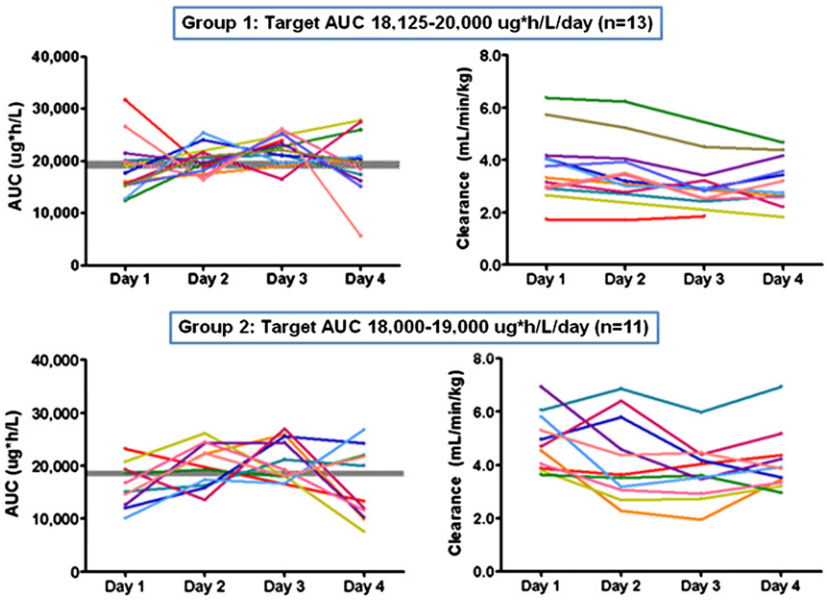

Figure 2. Intraindividual variability of busulfan pharmacokinetics. Even after daily dose adjustment, AUC was highly variable among the days.

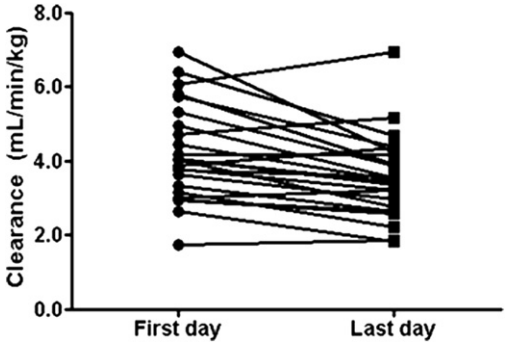

Figure 3. Clearances of the first and the last day. Clearances on the last day were significantly different from those of the first day ($P = .001$).

catalyzed by GST [30]. The interindividual variability could be partially explained by GST polymorphisms. Previous data are inconsistent. Patients with heterozygous variants of GSTA1 (GSTA1 *A/*B) appeared to show decreased clearance of busulfan in 2 studies, and 1 was a study with children [31,32]. Ansari et al. [33] suggested that i.v. busulfan clearance was affected by the GSTM1 genotype but not associated with the GSTA1 genotype. There were also studies suggesting that several GST polymorphisms could affect busulfan metabolism [34,35]. In contrast, data from 77 children suggested that GST polymorphisms were not associated with pharmacokinetic parameters of busulfan in pediatric patients [36]. Our study has its limitations because of the small number of patients, but the first day AUC had a tendency to be high in patients with the GSTA1 *A/*B or GSTT1 null genotype. Further studies with a sufficient number of patients will be needed to draw any conclusions.

In our study, there was a high intraindividual variability. The %CV of busulfan clearance of each individual ranged from 7.7%-38.7% (median 14.3%). We used fludarabine combined with busulfan. Fludarabine is commonly used in combination with busulfan as a part of conditioning regimens, and renal mechanisms play an important role in the elimination of fludarabine [37]. In a study of HSCT with oral busulfan and fludarabine, there were no changes in the pharmacokinetic parameters of fludarabine given before and after intake of busulfan, which implied that a clinically relevant busulfan-fludarabine drug interaction was unlikely [38]. To exclude the effect of other drugs as confounding factors, we used supportive drugs in the same manner during the period of busulfan infusion. We could not find the exact reason for the high intraindividual variability, but this means that even after the use of a targeted dose, it should be considered that residual variability in the actual exposure to busulfan might exist in some cases. Also, it could be recommended that intensive monitoring and dose adjustment are needed to overcome the high variability and to meet the total target AUC closely. In our study, we did the additional analysis if there was any correlation between the degree of intraindividual variability and patient outcome, but we could not find any relationship. In patients with high intraindividual variability, the total AUC was not much beyond the total target AUC because of the daily adjustment of busulfan dose. If the patient with high intraindividual variability received the same busulfan dose without TDM and dose adjustment, the patients could be over- or underexposed to busulfan. There are reports about the test dose of busulfan before HSCT to detect the fast and slow metabolizers [15,39]. The test dose could be useful to predict the interindividual variability and to avoid the extreme exposure of busulfan. However, intraindividual variability also should be considered even when the test dose is used.

We initially set up the total target AUC as 72,500-80,000 μg*h/L (17,661-19,488 μmol*min/L) based on the past literature of Bartelink et al. [40], in which event-free survival was optimal when the exposure of busulfan was 78 mg*h/L (95% confidence interval = 74 to 82 mg*h/L). However, total target AUC was reduced to 72,000-76,000 μg*h/L (17,539-18,513 μmol*min/L) after the observation of a high incidence of toxicity in the interim analysis. At the final analysis, VOD developed in 2 patients with total AUC of 79,469 and 81,367 μg*h/L (19,358 and

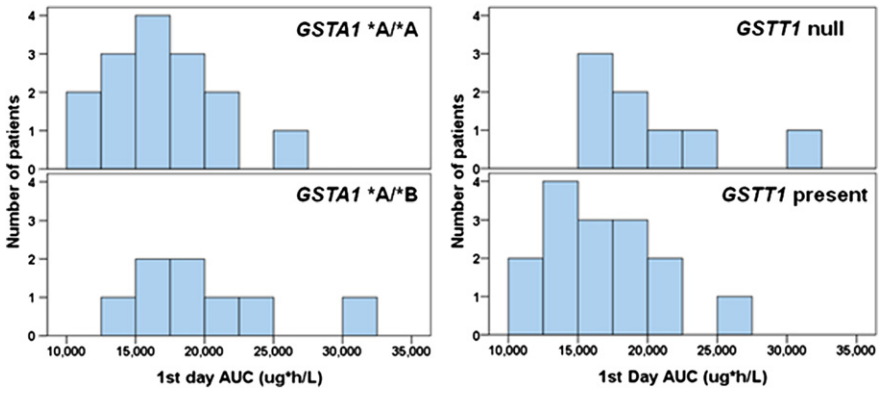

Figure 4. First day AUC according to the GST genotype. The first day AUC had a tendency to be high in patients with the GSTA1 *A/*B genotype or the GSTT1 null genotype.

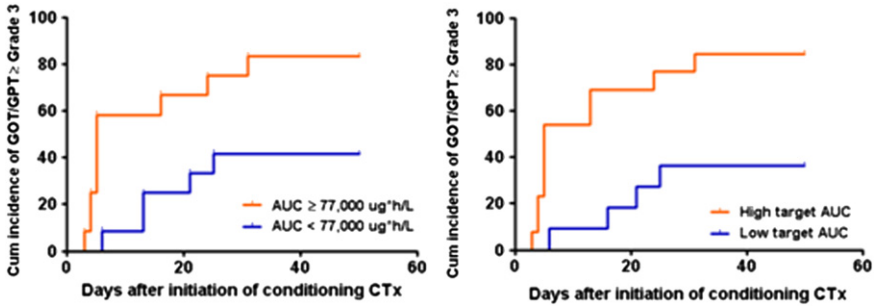

Figure 5. Liver toxicity according to the total and target AUC. Grade III/IV hepatic toxicities were more common in patients whose total AUC was over 77,000 μg*h/L ($P = .006$) and in patients of group 1 ($P = .007$).

19,821 μmol*min/L), and grade III/IV hepatic toxicities were more common in patients whose total AUC was over 77,000 μg*h/L (18,757 μmol*min/L). Those findings suggest that total target AUC <77,000 μg*h/L could be recommended to reduce toxicity and to improve the outcome of HSCT. O'Donnell and colleagues [41] in their phase 1 adult trial with busulfan/fludarabine/alemtuzumab found that it was the peak AUC rather than the average AUC that correlated best with sinusoidal obstruction syndrome/VOD, but there was no correlation between peak AUC and liver toxicity in our study.

Graft failure occurred with total target AUC <74,000 μg*h/L (18,026 μmol*min/L) in 3 patients who underwent CBT or T cell–depleted haploidentical transplantations. CBT and haploidentical transplantation are alternative means of HSCT in patients who do not have suitable siblings or unrelated matched donors, but graft failure is a limiting factor of those transplantations. To minimize the risk of graft failure, it is important to increase the infused CD34 cell dose, but it is not always possible. Also, the risk of GVHD could be increased with higher cell dose because of the increased T cells. Using the optimal dose of busulfan by TDM could be 1 of the ways to decrease graft failure while maximizing the antileukemic effect.

This study evaluated the pharmacokinetic characteristics of once-daily busulfan in pediatric patients. Regarding the study design, we limited the blood sampling time points for pharmacokinetic analysis to minimize the burden for children. The pharmacokinetics of busulfan has been well described by a single-compartment model in previous studies [10,42,43]. Therefore, blood samplings were taken at 4 points after busulfan infusion, and the pharmacokinetic parameters were calculated using a 1-compartment model. Consistent with previously reported studies, the individual time-plasma busulfan concentration profiles were best described by the 1-compartment model.

In conclusion, this study showed high inter- and intraindividual variability of busulfan pharmacokinetics in HSCT using a targeted busulfan/fludarabine regimen, which indicates the need for intensive monitoring and dose adjustment to improve the outcome of HSCT. We set up the target AUC on the fourth day as a (median value of the total target AUC range-cumulative AUC during 3 days) μg*h/L/day from group 2, and this method could be 1 of the means to meet the total target AUC more closely. Currently, we are performing a newly designed phase II study to decrease regimen-related toxicities and to reduce graft failure by setting an optimal target AUC based on this study.

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REFERENCES

1. Copelan EA, Bechtel TP, Avalos BR, et al. Busulfan levels are influenced by prior treatment and are associated with hepatic veno-occlusive disease and early mortality but not with delayed complications following marrow transplantation. *Bone Marrow Transplant*. 2001;27:1121-1124.
2. Dix SP, Wingard JR, Mullins RE, et al. Association of busulfan area under the curve with veno-occlusive disease following BMT. *Bone Marrow Transplant*. 1996;17:225-230.
3. Geddes M, Kangarloo SB, Naveed F, et al. High busulfan exposure is associated with worse outcomes in a daily i.v. busulfan and fludarabine allogeneic transplant regimen. *Biol Blood Marrow Transplant*. 2008;14:220-228.
4. Ljungman P, Hassan M, Bekassy AN, Ringden O, Oberg G. High busulfan concentrations are associated with increased transplant-related mortality in allogeneic bone marrow transplant patients. *Bone Marrow Transplant*. 1997;20:909-913.
5. McCune JS, Gibbs JP, Slattery JT. Plasma concentration monitoring of busulfan: does it improve clinical outcome? *Clin Pharmacokinet*. 2000;39:155-165.
6. Slattery JT, Clift RA, Buckner CD, et al. Marrow transplantation for chronic myeloid leukemia: the influence of plasma busulfan levels on the outcome of transplantation. *Blood*. 1997;89:3055-3060.
7. Madden T, de Lima M, Thapar N, et al. Pharmacokinetics of once-daily IV busulfan as part of pretransplantation preparative regimens: a comparison with an every 6-hour dosing schedule. *Biol Blood Marrow Transplant*. 2007;13:56-64.
8. Russell JA, Tran HT, Quinlan D, et al. Once-daily intravenous busulfan given with fludarabine as conditioning for allogeneic stem cell transplantation: study of pharmacokinetics and early clinical outcomes. *Biol Blood Marrow Transplant*. 2002;8:468-476.
9. Chiesa R, Cappelli B, Crocchiolo R, et al. Unpredictability of intravenous busulfan pharmacokinetics in children undergoing

hematopoietic stem cell transplantation for advanced beta thalassemia: limited toxicity with a dose-adjustment policy. *Biol Blood Marrow Transplant*. 2010;16:622-628.
10. Nath CE, Earl JW, Pati N, Stephen K, Shaw PJ. Variability in the pharmacokinetics of intravenous busulphan given as a single daily dose to paediatric blood or marrow transplant recipients. *Br J Clin Pharmacol*. 2008;66:50-59.
11. Tran H, Petropoulos D, Worth L, et al. Pharmacokinetics and individualized dose adjustment of intravenous busulfan in children with advanced hematologic malignancies undergoing allogeneic stem cell transplantation. *Biol Blood Marrow Transplant*. 2004;10:805-812.
12. Russell JA, Kangarloo SB. Therapeutic drug monitoring of busulfan in transplantation. *Curr Pharm Des*. 2008;14:1936-1949.
13. Bartelink IH, Bredius RG, Ververs TT, et al. Once-daily intravenous busulfan with therapeutic drug monitoring compared with conventional oral busulfan improves survival and engraftment in children undergoing allogeneic stem cell transplantation. *Biol Blood Marrow Transplant*. 2008;14:88-98.
14. Bleyzac N, Souillet G, Magron P, et al. Improved clinical outcome of paediatric bone marrow recipients using a test dose and Bayesian pharmacokinetic individualization of busulfan dosage regimens. *Bone Marrow Transplant*. 2001;28:743-751.
15. Kletzel M, Jacobsohn D, Duerst R. Pharmacokinetics of a test dose of intravenous busulfan guide dose modifications to achieve an optimal area under the curve of a single daily dose of intravenous busulfan in children undergoing a reduced-intensity conditioning regimen with hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2006;12:472-479.
16. Lemaistre JA, Bachier C, Smith B, Lemaistre C, Shaughnessy PJ. Once daily busulfan cyclophosphamide is well tolerated and effective as a preparative regimen for allogeneic hematopoietic stem cell transplant. *J Oncol Pharm Pract*. 2011 Jan 12 [Epub ahead of print].
17. Russell JA, Duan Q, Chaudhry MA, et al. Transplantation from matched siblings using once-daily intravenous busulfan/fludarabine with thymoglobulin: a myeloablative regimen with low nonrelapse mortality in all but older patients with high-risk disease. *Biol Blood Marrow Transplant*. 2008;14:888-895.
18. Ryu SG, Lee JH, Choi SJ, et al. Randomized comparison of four-times-daily versus once-daily intravenous busulfan in conditioning therapy for hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2007;13:1095-1105.
19. Zwaveling J, den Hartigh J, Lankester AC, Guchelaar HJ, Egeler RM, Bredius RG. Once-daily intravenous busulfan in children before stem cell transplantation: study of pharmacokinetics and early clinical outcomes. *Anticancer Drugs*. 2006;17:1099-1105.
20. Andersson BS, de Lima M, Thall PF, et al. Once daily i.v. busulfan and fludarabine (i.v. Bu-Flu) compares favorably with i.v. busulfan and cyclophosphamide (i.v. BuCy2) as pretransplant conditioning therapy in AML/MDS. *Biol Blood Marrow Transplant*. 2008;14:672-684.
21. Bornhauser M, Storer B, Slattery JT, et al. Conditioning with fludarabine and targeted busulfan for transplantation of allogeneic hematopoietic stem cells. *Blood*. 2003;102:820-826.
22. de Lima M, Couriel D, Thall PF, et al. Once-daily intravenous busulfan and fludarabine: clinical and pharmacokinetic results of a myeloablative, reduced-toxicity conditioning regimen for allogeneic stem cell transplantation in AML and MDS. *Blood*. 2004;104:857-864.
23. Lee JH, Choi J, Kwon KA, et al. Fludarabine-based myeloablative regimen as pretransplant conditioning therapy in adult acute leukemia/myelodysplastic syndrome: comparison with oral or intravenous busulfan with cyclophosphamide. *Korean J Hematol*. 2010;45:102-108.
24. Shaw PJ, Nath C, Berry A, Earl JW. Busulphan given as four single daily doses of 150 mg/m^2 is safe and effective in children of all ages. *Bone Marrow Transplant*. 2004;34:197-205.
25. Kang HJ, Shin HY, Choi HS, Ahn HS. Fludarabine, cyclophosphamide plus thymoglobulin conditioning regimen for unrelated bone marrow transplantation in severe aplastic anemia. *Bone Marrow Transplant*. 2004;34:939-943.
26. Kim MS, Kang HJ, Park HJ, et al. Development of multiplex PCR method for the analysis of glutathione S-transferase polymorphisms. *Mol Diagn Ther*. 2011.
27. Andersson BS, Madden T, Tran HT, et al. Acute safety and pharmacokinetics of intravenous busulfan when used with oral busulfan and cyclophosphamide as pretransplantation conditioning therapy: a phase I study. *Biol Blood Marrow Transplant*. 2000;6:548-554.
28. Cremers S, Schoemaker R, Bredius R, et al. Pharmacokinetics of intravenous busulfan in children prior to stem cell transplantation. *Br J Clin Pharmacol*. 2002;53:386-389.
29. Fernandez HF, Tran HT, Albrecht F, Lennon S, Caldera H, Goodman MS. Evaluation of safety and pharmacokinetics of administering intravenous busulfan in a twice-daily or daily schedule to patients with advanced hematologic malignant disease undergoing stem cell transplantation. *Biol Blood Marrow Transplant*. 2002;8:486-492.
30. McCune JS, Holmberg LA. Busulfan in hematopoietic stem cell transplant setting. *Expert Opin Drug Metab Toxicol*. 2009;5:957-969.
31. Johnson L, Orchard PJ, Baker KS, et al. Glutathione S-transferase A1 genetic variants reduce busulfan clearance in children undergoing hematopoietic cell transplantation. *J Clin Pharmacol*. 2008;48:1052-1062.
32. Kusama M, Kubota T, Matsukura Y, et al. Influence of glutathione S-transferase A1 polymorphism on the pharmacokinetics of busulfan. *Clin Chim Acta*. 2006;368:93-98.
33. Ansari M, Lauzon-Joset JF, Vachon MF, et al. Influence of GST gene polymorphisms on busulfan pharmacokinetics in children. *Bone Marrow Transplant*. 2010;45:261-267.
34. Elhasid R, Krivoy N, Rowe JM, et al. Influence of glutathione S-transferase A1, P1, M1, T1 polymorphisms on oral busulfan pharmacokinetics in children with congenital hemoglobinopathies undergoing hematopoietic stem cell transplantation. *Pediatr Blood Cancer*. 2010;55:1172-1179.
35. Kim SD, Lee JH, Hur EH, et al. Influence of GST gene polymorphisms on the clearance of intravenous busulfan in adult patients undergoing hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2011;17:1222-1230.
36. Zwaveling J, Press RR, Bredius RG, et al. Glutathione S-transferase polymorphisms are not associated with population pharmacokinetic parameters of busulfan in pediatric patients. *Ther Drug Monit*. 2008;30:504-510.
37. Adkins JC, Peters DH, Markham A. Fludarabine. An update of its pharmacology and use in the treatment of haematological malignancies. *Drugs*. 1997;53:1005-1037.
38. Bonin M, Pursche S, Bergeman T, et al. F-ara-A pharmacokinetics during reduced-intensity conditioning therapy with fludarabine and busulfan. *Bone Marrow Transplant*. 2007;39:201-206.
39. Takamatsu Y, Sasaki N, Eto T, et al. Individual dose adjustment of oral busulfan using a test dose in hematopoietic stem cell transplantation. *Int J Hematol*. 2007;86:261-268.
40. Bartelink IH, Bredius RG, Belitser SV, et al. Association between busulfan exposure and outcome in children receiving intravenous busulfan before hematologic stem cell transplantation. *Biol Blood Marrow Transplant*. 2009;15:231-241.
41. O'Donnell PH, Artz AS, Undevia SD, et al. Phase I study of dose-escalated busulfan with fludarabine and alemtuzumab as conditioning for allogeneic hematopoietic stem cell transplant: reduced clearance at high doses and occurrence of late sinusoidal obstruction syndrome/veno-occlusive disease. *Leuk Lymphoma*. 2010;51:2240-2249.
42. Shaw PJ, Scharping CE, Brian RJ, Earl JW. Busulfan pharmacokinetics using a single daily high-dose regimen in children with acute leukemia. *Blood*. 1994;84:2357-2362.
43. Nakamura H, Sato T, Okada K, et al. Population pharmacokinetics of oral busulfan in young Japanese children before hematopoietic stem cell transplantation. *Ther Drug Monit*. 2008;30:75-83.